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The Tritium Isotope Effect of *sn*-Glycerol 3-Phosphate Oxidase and the Effects of Clofenapate and *N*-(2-Benzoyloxyethyl)norfenfluramine on the Esterification of Glycerol Phosphate and Dihydroxyacetone Phosphate by Rat Liver Mitochondria

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1. Owing to a ^3H isotope effect, the mitochondrial *sn*-glycerol 3-phosphate oxidase (EC 1.1.99.5) had a mean activity which was 8.4 times less with *sn*-[2-^3H]- rather than with *sn*-[1-^{14}C]glycerol 3-phosphate as a substrate. 2. A method for measuring the simultaneous synthesis of lipid from glycerol phosphate and dihydroxyacetone phosphate in rat liver mitochondria is described. 3. The lipid synthesized by rat liver mitochondria from *sn*-[1-^{14}C]glycerol 3-phosphate was mainly phosphatidate and lysophosphatidate, whereas that synthesized from dihydroxy[1-^{14}C]acetone phosphate was mainly acyldihydroxyacetone phosphate. 4. Additions of NADPH facilitated the conversion of acyldihydroxyacetone phosphate into lysophosphatidate and phosphatidate. 5. Hydrazine (1.4mM) or KCN (1.4mM) inhibited the synthesis of lipids from dihydroxyacetone phosphate but not from glycerol phosphate. 6. Clofenapate (1–2.5mM) inhibited the synthesis of lipids from dihydroxyacetone phosphate but slightly stimulated synthesis from glycerol phosphate. 7. The methanesulphonate of *N*-(2-benzoyloxyethyl)norfenfluramine, at 0.25–0.75mM, inhibited lipid synthesis from both glycerol phosphate and dihydroxyacetone phosphate.

This paper is concerned with the oxidation of *sn*-glycerol 3-phosphate by rat liver mitochondria and the subsequent incorporation into lipids of the remaining glycerol phosphate and the dihydroxyacetone phosphate that is formed by oxidation.

From previous results (Manning & Brindley, 1972) it was suggested that the mitochondrial *sn*-glycerol 3-phosphate oxidase (EC 1.1.99.5) shows a marked discrimination against *sn*-[2-^3H]glycerol 3-phosphate. The ^3H isotope effect has now been measured directly by determining the simultaneous production of ^3H_2O and dihydroxy[1-^{14}C]acetone phosphate from a mixture of *sn*-[2-^3H]- and *sn*-[1-^{14}C]-glycerol 3-phosphate.

The mitochondrial preparation, which contained *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate after the oxidation reaction, was then allowed to synthesize lipids in the absence of further oxidation. It was established that lipids were synthesized from both *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate. The effects of KCN, hydrazine, clofenapate and the methane sulphonate of *N*-(2-benzoyloxyethyl)norfenfluramine (S1513) on these two pathways of lipid synthesis were then examined.

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Materials and Methods

Materials

Clofenapate [sodium 4-(4′-chlorophenyl)phenoxyisobutyrate] was a gift from Imperial Chemical Industries Ltd., Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K., and S1513 was given by Servier Laboratories Ltd., Percival House, Harrow, Middx. HA1 4HQ, U.K. NAD^+, NADH, NADPH and antimycin A were purchased from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K., and glycerol phosphate dehydrogenase (EC 1.1.1.8) was from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K. Other materials were described by Manning & Brindley (1972).

Measurement of sn-glycerol 3-phosphate oxidase activity

The rate of oxidation of *sn*-[2-^3H]glycerol 3-phosphate by rat liver mitochondria was determined by measuring the synthesis of ^3H_2O by a method based on that of Carnicero *et al.* (1972). The medium used for measuring the oxidation was that described by Manning & Brindley (1972) except that ATP was omitted. The initial concentration of *sn*-glycerol 3-phosphate was 1.57mM, containing 4.5μCi of *sn*-[2-^3H]glycerol 3-phosphate and 1μCi of *sn*-[1-^{14}C]glycerol 3-phosphate/ml. Samples (10μl) of the oxidation mixture were added to 1ml of 0.6M-

$HClO_4$ at 0°C. The solution was neutralized with 1ml of 0.6M-KOH at 0°C and the precipitate removed by centrifugation. The supernatant was diluted to 2.5ml with water and 1ml was distilled. Samples of the distillate were then assayed for ^3H_2O by using 10vol. of Triton X-100–xylene (1:2, v/v) containing 5.5g of 2,5-diphenyloxazole and 0.1g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre.

The rate of oxidation of *sn*-[1-^{14}C]glycerol 3-phosphate was determined from the rate of removal of *sn*-[1-^{14}C]glycerol 3-phosphate and the rate of appearance of dihydroxy[1-^{14}C]acetone phosphate (Manning & Brindley, 1972).

Measurement of glycerolipid synthesis from sn-[2-^3H]- and sn-[1-^{14}C]-glycerol 3-phosphate and dihydroxy-[1-^{14}C]acetone phosphate

At various time-intervals during the oxidation of *sn*-glycerol 3-phosphate, 100μl samples of the medium were pipetted into a second incubation medium, which contained antimycin A to prevent further oxidation. This esterification system contained, in a final volume of 0.5ml, 25mM-Tris (adjusted to pH7.4 with HCl), 5mM-potassium phosphate (pH7.4), 80mM-KCl, 5mM-$MgCl_2$, 2.5mM-ATP, 2mM-dithiothreitol, 1mM-EDTA (adjusted to pH7.4 with KOH), 50μM-CoA, 0.8mM-potassium palmitate, 5mM-NaF, 3mg of fatty acid-poor bovine serum albumin (Miles Laboratories Ltd., Stoke Poges, Slough SL2 4LY, U.K.), 2μg of rotenone, 1.5μg of oligomycin and 2μg of antimycin A. The initial concentration of *sn*-glycerol 3-phosphate was 315μM. NaF was added to prevent hydrolysis of phosphate esters by acid phosphatase. After incubation at 37°C for 15min the reaction was stopped with 40μl of conc. HCl and lipids were extracted by the method of Hajra *et al.* (1968). The rate of glycerolipid synthesis was constant throughout the 15min period. The incorporation of radioisotopes into lipid during the period of oxidation was less than 5% of that obtained after the second incubation. These former values were subtracted from the latter as blanks.

Characterization of newly synthesized lipids

Lipids were characterized by t.l.c. on plates of silica gel N (Machery, Nagel and Co., Düren, West Germany) made in 0.01M-Na_2CO_3 and developed with either chloroform–methanol–acetic acid–acetone–water (10:2:2:4:1, by vol.; Hajra & Agranoff, 1968), or chloroform–methanol–acetic acid–5% (w/v) sodium metabisulphite (25:10:3:1, by vol.; Hajra, 1968*b*). Lipids were recovered from the plates and ^{14}C was determined as described previously (Sánchez *et al.*, 1973). Alternatively lipids were extracted from the silica and the $^3H/^{14}C$ ratio was determined (Manning & Brindley, 1972).

Measurement of the concentration of dihydroxyacetone phosphate and sn-glycerol 3-phosphate in rat liver

Rats were killed by a blow on the neck and the livers frozen in liquid N_2 within 20s from the time of death. Duplicate samples of liver (approx. 0.5g) were homogenized in 2ml portions of ice-cold 6% (w/v) $HClO_4$. After centrifugation the precipitate was adjusted to pH5 with solid $KHCO_3$ and the $KClO_4$ removed by centrifugation. *sn*-Glycerol 3-phosphate dehydrogenase was used to assay dihydroxyacetone phosphate (Bücher & Hohorst, 1963) and *sn*-glycerol 3-phosphate (Hohorst, 1963).

Determination of protein

Protein concentrations were measured by a biuret method (Brindley & Hübscher, 1965).

Results and Discussion

Measurement of the ^3H isotope effect in the mitochondrial oxidation of sn-[2-^3H]glycerol 3-phosphate

It has previously been reported that *sn*-glycerol 3-phosphate oxidase exhibits a primary radioisotope effect during the oxidation of *sn*-[2-^{3}H]glycerol 3-phosphate (Plackett & Rodwell, 1970; Carnicero *et al.*, 1972; Manning & Brindley, 1972). Although the ^{3}H isotope effect was not measured directly it was suggested by Manning & Brindley (1972) that its magnitude was considerably greater than that reported by Carnicero *et al.* (1972). The present experiments were designed to give a direct measurement of the oxidation rate of *sn*-[2-^{3}H]glycerol 3-phosphate, on the basis of the formation of ^3H_2O. This was then compared with the rate of oxidation of *sn*-[1-^{14}C]-glycerol 3-phosphate, which was assumed to be identical with that of non-radioactive *sn*-glycerol 3-phosphate.

The radioisotope effect of the reaction can be defined as the ratio of the rate constants for the oxidation of unlabelled *sn*-glycerol 3-phosphate (k_H) and of *sn*-[2-^{3}H]glycerol 3-phosphate (k_T):

$$^3\text{H isotope effect} = \frac{k_H}{k_T} \qquad (1)$$

The amount of substrate present at any time is given by:

$$\ln(1-f) = kt \qquad (2)$$

where k is the appropriate rate constant and f is the fraction of substrate oxidized.

Therefore at any time-interval:

$$^3\text{H isotope effect} = \frac{k_H}{k_T} = \frac{\ln(1-f_H)}{\ln(1-f_T)} \qquad (3)$$

where f_H and f_T are the fractions of unlabelled and *sn*-[2-^{3}H]glycerol 3-phosphate respectively.

When f_H and f_T are small relative to 1, then:

$$^3\text{H isotope effect} \approx \frac{f_H}{f_T} \quad (4)$$

The solutions to eqns. (3) and (4) are shown in Fig. 1, giving a ^{3}H isotope effect of between 7.7 and 7.8. The mean ^{3}H isotope effect from four different preparations of mitochondria was 8.4±0.4 (S.D.). This value is greater than the value of 2.17, which can be calculated from the results of Carnicero *et al.* (1972), and the discrepancy may be attributed to differences in experimental conditions.

The consequence of the large ^{3}H isotope effect demonstrated in Fig. 1 is that the specific radioactivity of *sn*-glycerol 3-phosphate, with respect to 2-^{3}H, increases during mitochondrial oxidation (Fig. 2). This means that the radioisotope effect of *sn*-glycerol 3-phosphate oxidation must be taken into account when [2-^{3}H]glycerol or *sn*-[2-^{3}H]glycerol 3-phosphate are used to investigate metabolic reactions, such as the synthesis of lipids.

Measurement of the simultaneous incorporation of sn-[1-^{14}C]glycerol 3-phosphate and dihydroxy-[1-^{14}C]acetone phosphate into lipids by rat liver mitochondria

The incubation system used in the above experiments to measure the radioisotope effect of *sn*-glycerol 3-phosphate oxidase was also used in a second series of experiments to measure the simultaneous synthesis of glycerolipids from *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate. At various time-intervals during the oxidation period samples of the oxidation mixture were withdrawn and added to an esterification system, which contained antimycin A to prevent further oxidation (see the Materials and Methods section). By this procedure it was possible to add a wide variety of concentrations of *sn*-glycerol 3-phosphate (labelled with ^{3}H and ^{14}C) and dihydroxyacetone phosphate (labelled solely with ^{14}C) to the esterification system.

As expected from previous experiments (Manning & Brindley, 1972) the ratio of d.p.m. of 2-^{3}H/d.p.m. of 1-^{14}C (2-^{3}H/1-^{14}C ratio) in *sn*-glycerol 3 phosphate rose markedly as the oxidation proceeded (Fig. 2). This is a direct result of the ^{3}H isotope effect of the *sn*-glycerol 3-phosphate oxidase (Fig. 1). In contrast, the 2-^{3}H/1-^{14}C ratio in lipid was considerably lower than that in the precursor *sn*-glycerol 3-phosphate at a corresponding time of oxidation. The difference between these values is assumed to be due to the simultaneous incorporation of dihydroxy-[1-^{14}C]acetone phosphate into lipid. At zero time of oxidation the 2-^{3}H/1-^{14}C ratio of the glycerolipid produced during the esterification period was identical with that in the precursor *sn*-glycerol 3-phosphate. This demonstrates that the acyltransferases, which operate during the esterification process, do not exhibit significant ^{3}H isotope effects.

The contributions of the glycerol phosphate and

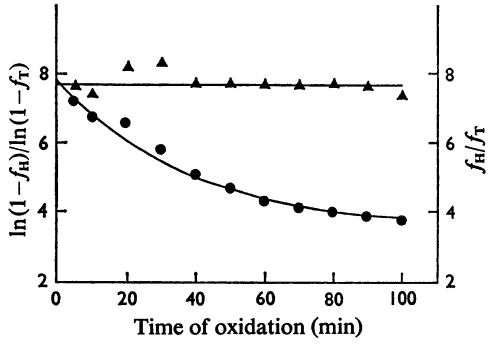

Fig. 1. *Measurement of the ^{3}H isotope effect of the sn-glycerol 3-phosphate oxidase of rat liver mitochondria*

The fractional oxidation of *sn*-[2-^{3}H]glycerol 3-phosphate (f_T) and *sn*-[1-^{14}C]glycerol 3-phosphate (f_H) were measured at various time-intervals of incubation as described in the Materials and Methods section. The relationship of f_H/f_T (●) and ln(1−f_H)/ln(1−f_T) (▲) is illustrated.

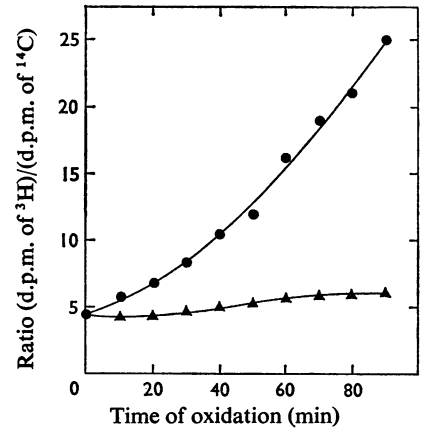

Fig. 2. *Effect of mitochondrial oxidation on the 2-^{3}H/1-^{14}C ratio in the remaining sn-glycerol 3-phosphate and in the newly synthesized lipid*

The incubation systems are described in the Materials and Methods section. The 2-^{3}H/1-^{14}C ratio in *sn*-glycerol 3-phosphate (●) and in the total lipids (▲) synthesized at the corresponding time of oxidation is shown.

dihydroxyacetone phosphate pathways to lipid synthesis can be calculated from the 2-^{3}H/1-^{14}C ratio in *sn*-glycerol 3-phosphate and the rate of lipid synthesis at the corresponding time of oxidation. This is done by using eqns. (1) and (3), which were described by Manning & Brindley (1972). However, the 2-^{3}H/1-^{14}C ratio of *sn*-glycerol 3-phosphate does not change during the period of esterification, and therefore it is

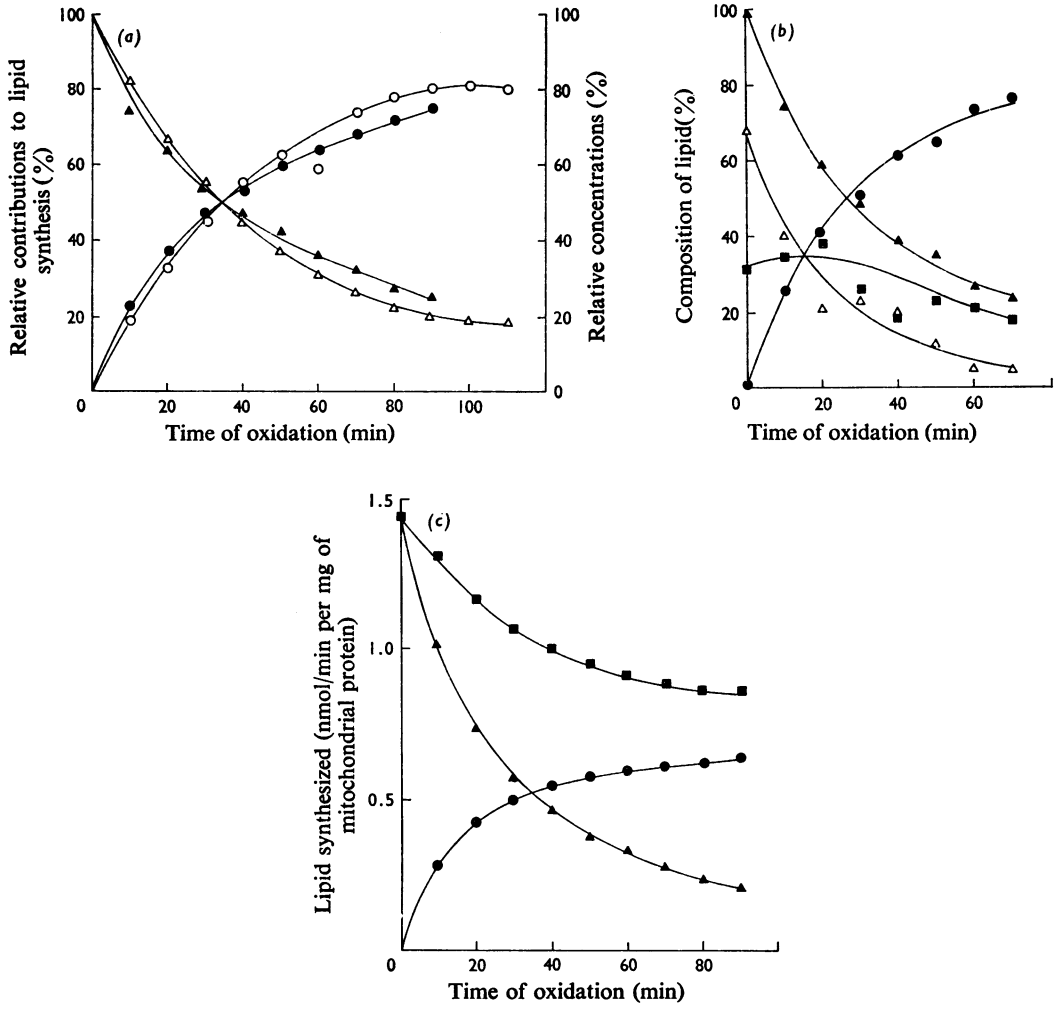

Fig. 3. *Simultaneous synthesis of glycerolipids from sn-*[1-^{14}*C*]*glycerol 3-phosphate and dihydroxy*[1-^{14}*C*]*-acetone phosphate by rat liver mitochondria*

At various time-intervals during the oxidation reaction samples of the medium were pipetted into a second incubation mixture in which glycerolipid synthesis could be studied in the absence of further oxidation (see the Materials and Methods section). Fig. 3(*a*) shows, in terms of relative contributions and concentrations of glycerol phosphate and dihydroxyacetone phosphate, the disappearance of *sn*-[1-^{14}C]glycerol 3-phosphate (△), the appearance of dihydroxy[1-^{14}C]acetone phosphate (○), the activity of the glycerol phosphate pathway (▲) and the activity of the dihydroxyacetone phosphate pathway (●). Fig. 3(*b*) shows the percentage of [^{14}C]glycerolipid isolated as acyldihydroxyacetone phosphate (●), phosphatidate (■), lysophosphatidate (△) and phosphatidate plus lysophosphatidate (▲). The contribution of neutral lipid was less than 5%. In Fig. 3(*c*) the rate of lipid synthesis by the glycerol phosphate pathway (▲) and dihydroxyacetone phosphate pathway (●) plus the total synthesis of lipid (■) are shown as a function of the time for which the mitochondria were allowed to oxidize *sn*-glycerol 3-phosphate.

not necessary to integrate in order to solve the equations.

It is suggested that these calculations give a valid measurement of the relative activities of the two pathways from the following observations.

(a) The relative activities calculated for the two pathways closely follow the relative concentrations of their respective precursors in the incubation system (Fig. 3*a*).

(b) Analysis of the newly synthesized lipids shows that the calculated activity of the dihydroxyacetone phosphate pathway (Fig. 3*a*) can be attributed to the synthesis of acyldihydroxyacetone phosphate (Fig. 3*b*) which was labelled solely with ^{14}C. Additions of 50–100 μM-NADPH to the esterification system resulted in the disappearance of acyldihydroxy[1-^{14}C]-acetone phosphate, and the appearance of additional [^{14}C]lysophosphatidate and [^{14}C]phosphatidate.

(c) The calculated activity of the glycerol phosphate pathway (Fig. 3*a*) can be attributed to the synthesis of lysophosphatidate and phosphatidate (Fig. 3*b*). The 2-^{3}H/1-^{14}C ratio of these lipids was essentially the same as that of the precursor *sn*-glycerol 3-phosphate at a corresponding time of oxidation.

(d) KCN and hydrazine are known to inhibit the synthesis of lipids from dihydroxyacetone phosphate because of their interactions with carbonyl groups (Hajra *et al.*, 1968). These reagents did not affect the activity of the glycerol phosphate pathway (Table 1, A) but decreased synthesis via dihydroxyacetone phosphate. This inhibition was accounted for by a decreased synthesis of acyldihydroxyacetone phosphate and a consequent increase in the 2-^{3}H/1-^{14}C ratio of the total lipid fraction.

(e) Addition of up to 300nmol of non-radioactive dihydroxyacetone phosphate to the esterification system, at 36min of oxidation, resulted in a proportional increase of the 2-^{3}H/1-^{14}C ratio of glycerolipid. This was probably caused by radioisotope dilution of the dihydroxy[1-^{14}C]acetone phosphate. The presence of the exogenous dihydroxyacetone phosphate had no effect on ^{3}H incorporation, indicating that this compound did not compete with *sn*-glycerol 3-phosphate for esterification in this system. This confirms the results of Hajra (1968*a*), who found that the addition of *sn*-glycerol 3-phosphate did not decrease the incorporation of dihydroxyacetone [^{32}P]phosphate into glycerolipid in a mitochondrial preparation from guinea-pig liver.

The rates of incorporation into lipids of dihydroxyacetone phosphate and glycerol phosphate after various times of oxidation are shown in Fig. 3(*c*). These results confirm previous evidence for the independent synthesis of lipids from dihydroxyacetone

Table 1. *Effects of hydrazine, KCN, clofenapate and S1513 on the synthesis of glycerolipids from sn-glycerol 3-phosphate and dihydroxyacetone phosphate by rat liver mitochondria*

Samples (100 μl) of the oxidation mixture were taken after 40min (System A) or 30min (Systems B and C) in order to study glycerolipid synthesis (see the Materials and Methods section). The degree of *sn*-glycerol 3-phosphate oxidation varied in three different preparations of mitochondria (55–66%, System A; 45–56%, Systems B and C). The rates of total lipid synthesis were respectively 0.756±0.207 (S.D.) and 0.825±0.214 (S.D.) nmol/min per mg of mitochondrial protein. The activity of each pathway, calculated as described in the text, was defined as 100% in the absence of additions (control). The additions are indicated in the table and the relative activities (±S.D.) of the two pathways are shown. Numbers in parentheses refer to the number of different mitochondrial preparations used. The significance of differences was calculated by using a paired *t* test.

		Relative activity (%)		Significance of difference between		
System	Additions	(1) Glycerol phosphate pathway	(2) Dihydroxyacetone phosphate pathway	(1) and (2)	Control and (1)	Control and (2)
A	1.4mM-Hydrazine	104±3 (3)	60±6 (3)	<0.005	<0.050	<0.005
	1.4mM-KCN	106±12 (3)	53±8 (3)	<0.005	<0.300	<0.005
B	0.25mM-S1513	49±5 (3)	72±9 (3)	<0.010	<0.003	<0.025
	0.50mM-S1513	31±1 (4)	44±7 (4)	<0.025	<0.001	<0.005
	0.75mM-S1513	26±1 (3)	31±2 (3)	<0.025	<0.001	<0.001
C	1.0mM-Clofenapate	125±12 (3)	70±32 (3)	<0.025	<0.050	<0.15
	2.5mM-Clofenapate	122±27 (4)	36±42 (4)	<0.010	<0.150	<0.05
	5.0mM-Clofenapate	96±70 (3)	39±35 (3)	<0.10	<0.475	<0.05

phosphate and glycerol phosphate by rat liver mitochondria (Hajra, 1968*a*; LaBelle & Hajra, 1972).

Effects of clofenapate and S1513 on the synthesis of glycerolipids by rat liver mitochondria

It has recently been shown that two classes of drugs inhibit glycerolipid synthesis *in vitro*. First, *p*-chlorophenoxyisobutyrate, a hypolipidaemic agent, has been shown to inhibit *sn*-glycerol 3-phosphate esterification in cell-free preparations from rat liver (Scott & Hurley, 1969; Adams *et al.*, 1971; Lamb & Fallon, 1972; Fallon *et al.*, 1972). A derivative of *p*-chlorophenoxyisobutyrate, clofenapate, proved to be more active in decreasing serum triglyceride and lipoprotein concentrations (Craig, 1972) and in inhibiting the hepatic esterification of *sn*-glycerol 3-phosphate *in vitro* (Brindley *et al.*, 1973).

Fenfluramine [2-ethylamino-1-(3-trifluoromethylphenyl)propane], which is used in treating obesity, inhibits the synthesis of glycerides in preparations from rat and human liver (Marsh & Bizzi, 1972; Brindley *et al.*, 1973), rat intestine (Dannenburg *et al.*, 1973) and human adipose tissue (Wilson & Galton, 1971). A derivative of fenfluramine, S1513, is more active in cell-free preparations than fenfluramine itself (Wilson & Galton, 1971; Brindley *et al.*, 1973).

The effects of clofenapate and S1513 on the synthesis of lipids from *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate were investigated and the results are presented in Table 1, B and C. S1513 was considerably more potent than clofenapate, on a molar basis, in inhibiting lipid synthesis. S1513 inhibited both pathways of lipid synthesis, though it produced a slightly greater percentage inhibition in the glycerol phosphate compared with the dihydroxyacetone phosphate pathway (Table 1, B). Inhibition of the latter pathway was accompanied by a disappearance of acyldihydroxyacetone phosphate.

In contrast, at similar percentage inhibitions of the dihydroxyacetone phosphate pathway clofenapate had little effect on lipid synthesis from *sn*-glycerol 3-phosphate. With 1mM-clofenapate, a small but significant stimulation in the acylation of *sn*-glycerol 3-phosphate was observed (Table 1, C). This may indicate that the acyltransferases for glycerol phosphate and dihydroxyacetone phosphate are different. Higher concentrations of clofenapate were able to inhibit this reaction (Brindley *et al.*, 1973), but in the present experiments the effects of clofenapate were less reproducible, with respect to concentration, than were those of S1513. These discrepancies may have been caused by solubility problems and differences in the physical form of clofenapate in the assay systems. The results from Table 1 indicate that, at least with rat liver mitochondria, the two classes of drugs may have different abilities to inhibit glycerolipid synthesis via the glycerol phosphate and dihydroxyacetone phosphate pathways.

One of the theories about the hypolipidaemic effect of clofibrate, *p*-chlorophenoxyisobutyrate and clofenapate is that these compounds indirectly stimulate the mitochondrial *sn*-glycerol 3-phosphate oxidase through thyroxine (Platt & Thorp, 1966; Westerfeld *et al.*, 1968). The inhibition of lipid synthesis could then be explained in terms of a decreased pool of *sn*-glycerol 3-phosphate (Pereira & Holland, 1970; Azarnoff, 1971). However, it has been stated recently that the effect of *p*-chlorophenoxyisobutyrate is not mediated through an enhanced effect of thyroxine on liver (Westerfeld *et al.*, 1972; Elwood *et al.*, 1972). In addition, the work performed on liver, heart and adipose tissue indicates that the intracellular concentrations of *sn*-glycerol 3-phosphate alone are unlikely to regulate the synthesis of glycerolipids (Denton & Randle, 1967; Denton & Halperin, 1968; Zakim & Herman, 1968; Saggerson & Greenbaum, 1970*a*,*b*; Adams *et al.*, 1971). The tissue concentrations of *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate reported by most authors lie in the ranges 130–900 and 17–30nmol/g wet wt. of rat liver respectively (Tzur *et al.*, 1964; Zakim & Herman, 1968; Veech *et al.*, 1970; Greenbaum *et al.*, 1971; McGarry & Foster, 1971; Kähönen *et al.*, 1972). In six rats of the strain used in these experiments we estimated the concentration of *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate to be 494 ± 28.8 (S.E.M.) and 27.5 ± 1.1 (S.E.M.)nmol/g wet wt. of liver respectively. Therefore even a small increase in the rate of oxidation could increase the concentration of dihydroxyacetone phosphate available for esterification.

Experiments with rat liver slices indicate that 50–60% of the synthesized glycerolipid could be derived via the dihydroxyacetone phosphate pathway (Manning & Brindley, 1972). If clofenapate were to increase the flux of carbon atoms into the dihydroxyacetone phosphate pool it might be expected to increase rather than decrease the synthesis of glycerolipids. Therefore the ability of clofenapate to inhibit enzymes of the dihydroxyacetone phosphate pathway might be significant in its hypolipidaemic action, although it is not proved that the results shown in Table 1 contribute to the therapeutic action of this drug.

It is becoming evident that the synthesis of glycerolipids from dihydroxyacetone phosphate may be of considerable physiological importance. The activity of this pathway, in addition to that of the glycerol phosphate pathway, should be taken into account when the control of glycerolipid synthesis is discussed.

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